Award Number: W81XWH-10-1-0585

TITLE: A Gene Expression Profile of BRCAness That Predicts for Responsiveness to Platinum and PARP Inhibitors

PRINCIPAL INVESTIGATOR: Panagiotis Konstantinopoulos

CONTRACTING ORGANIZATION: Dana Farber Cancer Institute
Boston, MA 02115

REPORT DATE: AUGUST 2014

TYPE OF REPORT: Annual Report

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE *(DD-MM-YYYY)*	**2. REPORT TYPE**	**3. DATES COVERED** *(From - To)*
AUGUST 2014	Annual Report	15 July 2013 - 14 July 2014

4. TITLE AND SUBTITLE A Gene Expression Profile of BRCAness That Predicts for Responsiveness to Platinum and PARP Inhibitors

5a. CONTRACT NUMBER

5b. GRANT NUMBER
W81XWH-10-1-0585

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S) Panagiotis Konstantinopoulos

E-Mail: panagiotis_konstantinopoulos@dfci.harvard.edu

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
Dana Farber Cancer Institute
Boston, MA, 02215

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT

The promise of PARP-inhibitors in the management of epithelial ovarian cancer is tempered by the fact that approximately 50% of patients with homologous recombination (HR)-proficient tumors do not respond well to these agents. Using a bioinformatics approach, we identified that heat shock protein 90 inhibitors(HSP90i) may suppress HR and thus revert HR-proficient to HR-deficient tumors. Analysis of publicly available gene expression data showed that exposure of HR-proficient cancer cell lines to HSP90i 17-AAG(17-allylamino-17-demethoxygeldanamycin) downregulated HR, ATM and Fanconi Anemia pathways. In HR-proficient EOC cells, 17-AAG suppressed HR as assessed using the RAD51 foci formation assay and this was further confirmed using the Direct Repeat-GFP reporter assay. Furthermore, 17-AAG downregulated BRCA1 and/or RAD51 protein levels, and induced significantly more γH2AX activation in combination with olaparib compared to olaparib alone. Finally, sublethal concentrations of 17-AAG sensitized HR-proficient EOC lines to olaparib and carboplatin but did not affect sensitivity of the HR-deficient OVCAR8 line arguing that the 17-AAG mediated sensitization is dependent on suppression of HR. These results provide a preclinical rationale for using a combination of olaparib/17-AAG in HR-proficient EOC.

15. SUBJECT TERMS
ovarian cancer, homologous recombination, brcaness, PARP inhibitors, platinum analogues, gene expression profiling, HSP90 inhibitors, Keap1-Nrf2 pathway

16. SECURITY CLASSIFICATION OF:			**17. LIMITATION OF ABSTRACT**	**18. NUMBER OF PAGES**	**19a. NAME OF RESPONSIBLE PERSON**
a. REPORT	**b. ABSTRACT**	**c. THIS PAGE**			USAMRMC
U	U	U	UU	14	**19b. TELEPHONE NUMBER** *(include area code)*

Table of Contents

1. INTRODUCTION

Patients with BRCA1/2-associated EOCs exhibit improved overall survival and high sensitivity to double strand DNA break inducing agents due to an underlying defect in DNA repair via HR. However, it is increasingly recognized that a subset of patients with sporadic EOCs also exhibit defective HR caused by mechanisms that are unrelated to germline BRCA1 or BRCA2 mutations. These tumors may behave similarly to BRCA1/2-mutated EOCs and are commonly referred to as having a "BRCAness" phenotype. Identifying tumors with a BRCAness phenotype is of increased clinical importance not only due to the advent of PARP-inhibitors but also because patients with this phenotype may need to be managed differently than the remaining patients. We have developed a 60-gene expression profile that may identify tumors with a BRCAness phenotype and may also be used to identify compounds that can enhance responsiveness to platinum and PARP-inhibitors. In this annual progress report of the work performed from 7/15/2013 to 7/14/2014, we discuss our findings regarding using this profile to identify HSP90-inhibitors as drugs that sensitize to platinum and PARP-inhibitors and present our in vitro data that validates this strategy.

2. KEYWORDS

Ovarian cancer, Homologous recombination, BRCAness, Gene expression profiling, PARP inhibitors, Platinum analogues, HSP90 inhibitors, 17-AAG, RAD51 foci assay, Connectivity Map, Platinum Resistance

3. ACCOMPLISHMENTS

- **What were the major goals of the project?**

The major goals of this project during this period was to evaluate whether the compounds identified by the Connectivity Map can reverse PARP resistance in vitro, and to investigate the mechanism for this effect.

- **What was accomplished under these goals?**

We have fully accomplished the goals of this project during this period. Specifically, using a bioinformatics approach in epithelial ovarian cancer models, we identified that HSP90 inhibitors may be functionally associated with induction of defective HR and reversion of HR proficient to HR-deficient tumors (Supplementary Figure 1). Furthermore, analysis of publicly available gene expression data showed that exposure of HR-proficient cancer cell lines to the HSP90 inhibitor 17-AAG (17-allylamino-17-demethoxygeldanamycin) statistically significantly downregulated HR ($p<0.005$), ATM ($p=0.015$) and Fanconi Anemia ($p<0.005$) pathways, and downregulated the expression levels of several genes of these pathways.

Importantly, in HR-proficient ovarian cancer cells, sublethal doses of HSP90i 17-AAG (as determined in the dose responses curves in each cell line shown in supplementary Figure 2) suppressed HR as assessed using the RAD51 foci formation after ionizing radiation (IR) assay and using the Direct Repeat-GFP (DR-GFP) reporter assay (Figure 1).

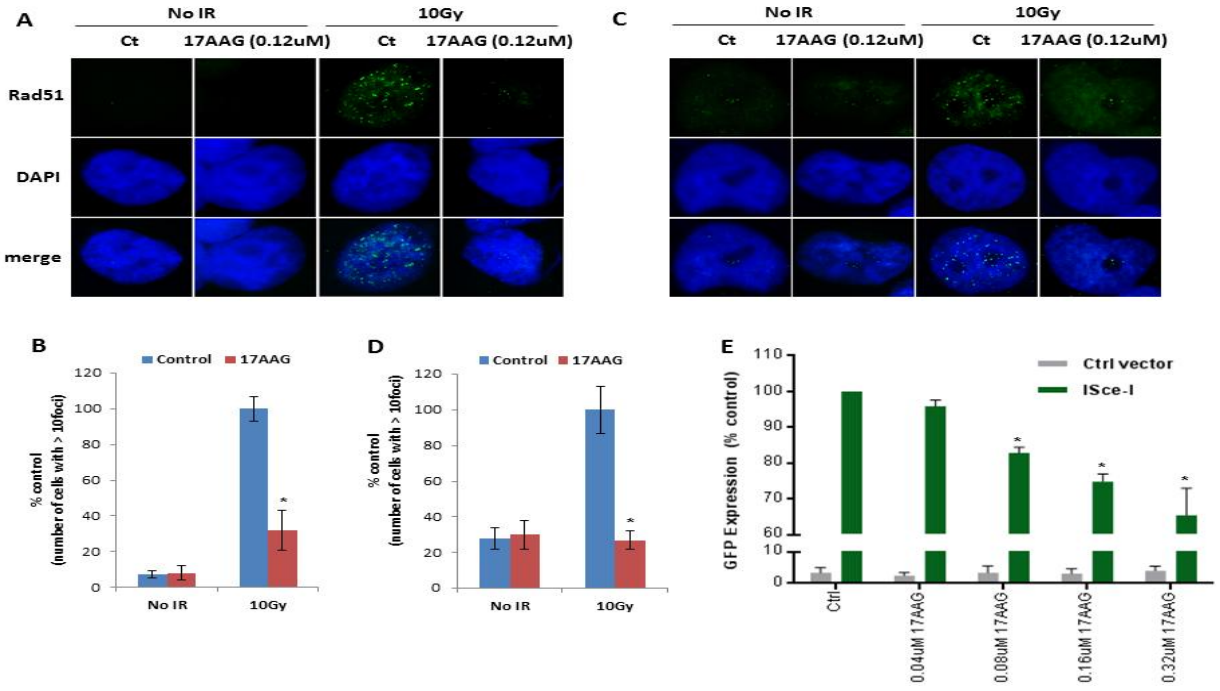


Figure 1: 17-AAG downregulates HR-mediated DSB repair. (A-D) Analysis of HR-mediated repair by RAD51 focus formation. 36M2 cells (A and B) or SKOV3 cells (C and D) were treated with 17-AAG or vehicle control for 24 hrs, stained for RAD51 (green) and DAPI (blue) 6 h after exposure to IR. (E) Measurement of HR-mediated repair of an I-SceI induced site specific DSB. Cells carrying a single copy of the recombination substrate (DR-GFP) were treated with indicated concentrations of 17-AAG or vehicle control for 24 hrs before transfection with I-SceI or control vector.

Additionally, sublethal doses of 17-AAG downregulated protein levels of BRCA1 and/or RAD51, and induced significantly more γH2AX activation in combination with olaparib compared to olaparib alone in HR proficient ovarian cancer cells (Figure 2).

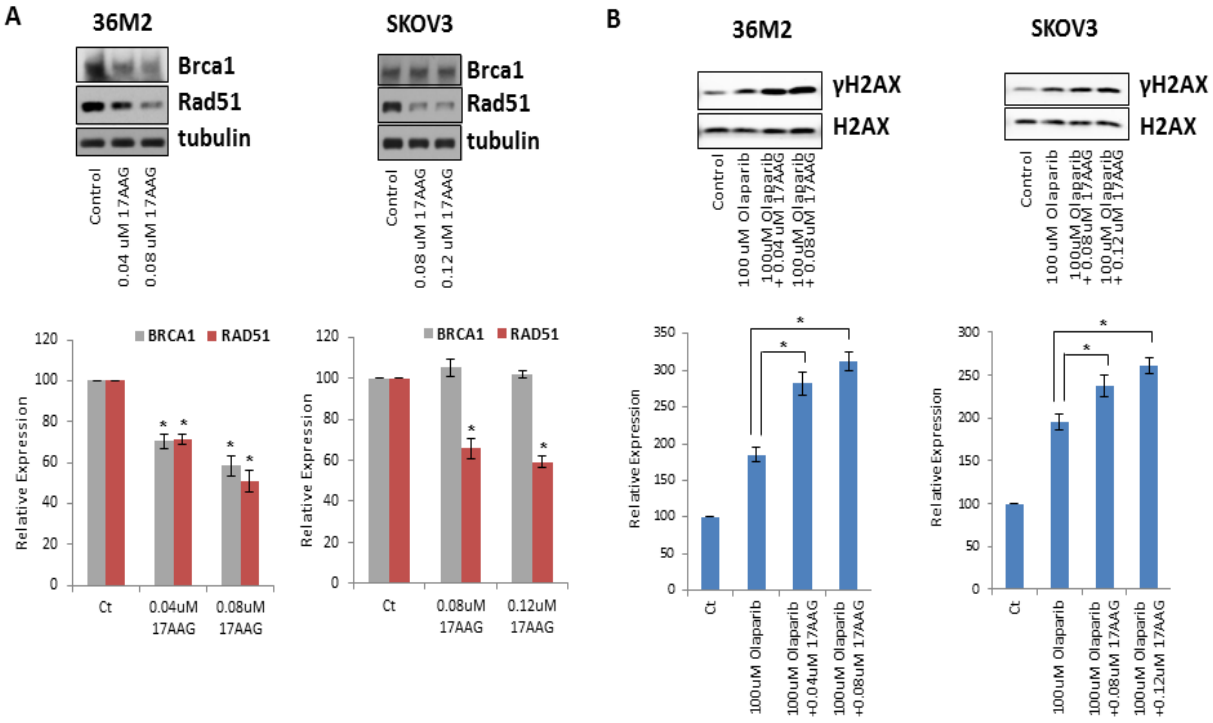


Figure 2: 17-AAG downregulates BRCA1 and/or RAD51. (A) Indicated cells were treated with 17-AAG or vehicle control for 24 hrs and washed off before subjected to immunoblotting in another 24 hrs. Cell lysates were analyzed by immunoblot for BRCA1 or RAD51 (B) γ-H2AX accumulation after treatment with olaparib ± 17-AAG. Indicated cells were treated with olaparib ± 17-AAG for 24 hrs before evaluation of γ-H2AX by immunoblotting.

Importantly, sublethal concentrations of 17-AAG sensitized HR proficient ovarian cancer lines to both olaparib and carboplatin (Figure 3).

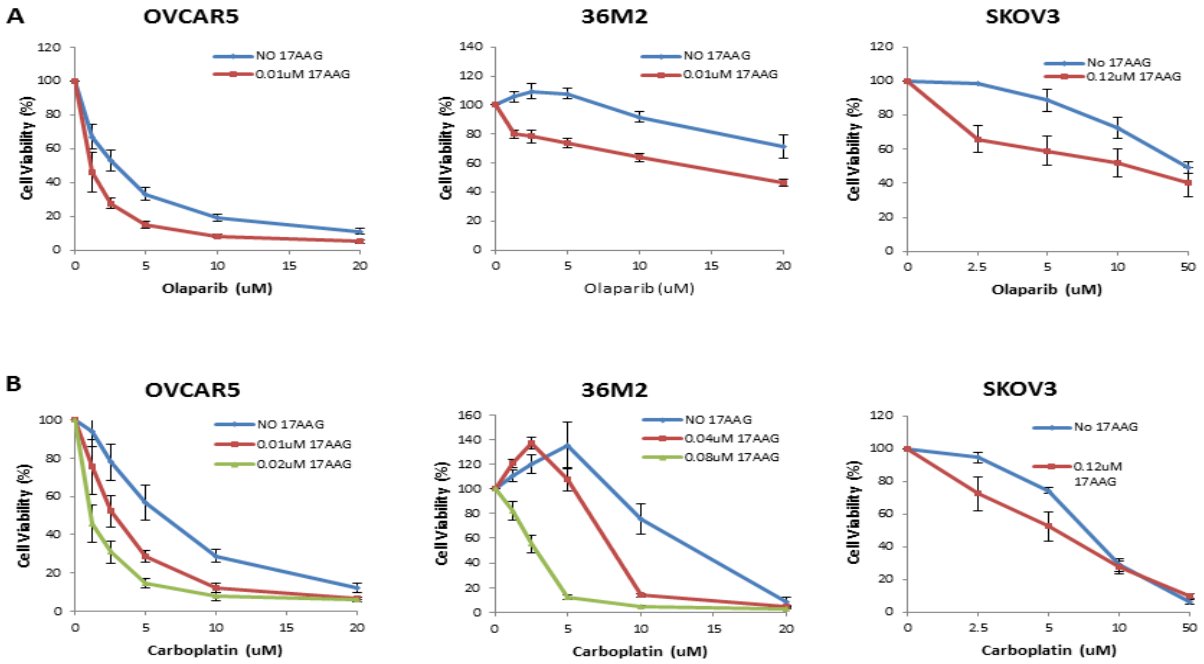


Figure 3: 17-AAG sensitizes HR-proficient cells to olaparib and carboplatin. (A and B) Luminescence-based viability assay in HR-proficient cells with olaparib or carboplatin. Cells were plated onto a 96-well plate at 1000 cells/well density and treated with indicated concentrations of PARP inhibitor, olaparib (A) or platinum drug, carboplatin (B) on the following day. Viability was tested by using CellTiter Glo (Promega) in 5 days. Curves were generated from 3 independent experiments.

Apart from HR proficient cell lines, we also evaluated the effect of 17-AAG in HR deficient ovarian cancer cells, specifically the OVCAR8 cell line which harbors almost undetectable levels of BRCA1 protein (Figure 4A) and very low levels of BRCA1 transcript (Figure 4B) compared to 36M2 ovarian cancer line. As shown in Figure 4C, sublethal concentrations of 17-AAG did not sensitize OVCAR8 cells to olaparib or carboplatin. This finding strongly suggests that the 17-AAG induced sensitization to olaparib and carboplatin is related to suppression of HR and not due to another (HR-independent) mechanism. Although our data suggest that 17-AAG suppresses HR and sensitizes to olaparib and carboplatin in vitro, we would like to recognize that 17-AAG-induced sensitization to platinum and PARP-inhibitors need to be further confirmed using in vivo models. In conclusion, sublethal concentrations of the HSP90i 17-AAG suppress HR and enhance sensitivity of HR proficient ovarian cancer cells to platinum and PARPis. These results provide a preclinical rationale for using a combination of 17-AAG and olaparib and/ or carboplatin in EOCs that are HR proficient either at baseline or at the time of development of platinum or PARPi resistance which was a major goal of our project.

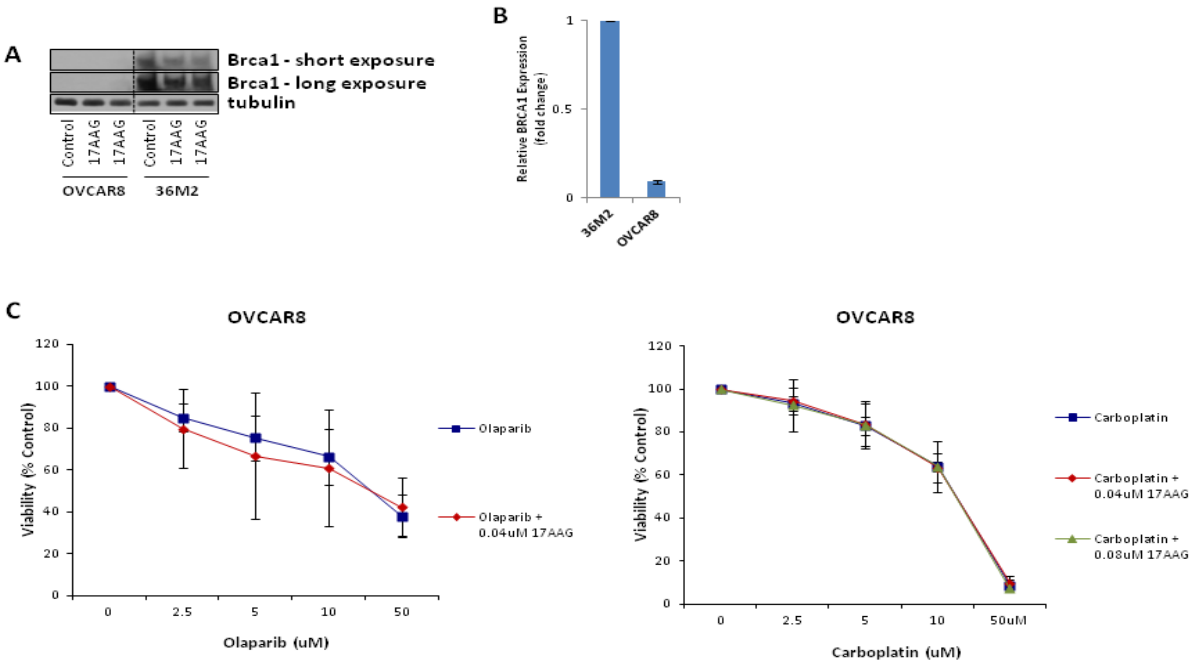


Figure 4. 17-AAG does not sensitize HR-deficient cells to olaparib or carboplatin. (A and B) Validation of undetectable levels of BRCA1 expression in OVCAR8 cells. OVCAR8 cells were analyzed for BRCA1 expression by immunoblotting (A) and qRT-PCR (B) compared to BRCA1-proficient 36M2 cells. (C) Viability assay in HR-deficient cells with PARP inhibitor or platinum drug. Viability assay was done in the same way as in Figure 3.

- **What opportunities for training and professional development has the project provided?**

Nothing to report.

- **How were the results disseminated to communities of interest?**

Nothing to report.

- **What do you plan to do during the next reporting period to accomplish the goals?**

In the next funding period, we plan to investigate whether the effect that we observed for 17-AAG is also applicable for other HSP90-inhibitors and we plan validate whether the mechanism for that effect is through abrogation of the HR pathway. Furthermore, we plan to determine the

reproducibility of the BRCAness profile when using the DASL mRNA assay in a cohort of FFPE ovarian cancer specimens with known clinical outcome and platinum responsiveness.

4. IMPACT

- **What was the impact on the development of the principal discipline(s) of the project?**

The promise of PARP inhibitors in the management of ovarian cancer is tempered by the fact that HR-proficient cancers do not respond well to these agents, suggesting that approximately 50% of ovarian cancer patients (i.e. those without HR alterations) do not benefit from this novel class of drugs. Combination of PARPis with agents that inhibit HR may represent an effective strategy to sensitize HR proficient tumors to PARPis and thus potentially expand use of these agents beyond patients with HR deficient EOCs. Our findings that sublethal concentrations of the HSP90i 17-AAG suppress HR and enhance sensitivity of HR proficient ovarian cancer cells to platinum and PARPis provide a preclinical rationale for using a combination of 17-AAG and olaparib and/ or carboplatin in ovarian cancers that are HR proficient either at baseline or at the time of development of platinum or PARPi resistance. This can have a significant impact on patients who develop resistance to PARP-inhibitors or platinum analogues as the combination of 17-AAG/PARP-inhibitors or 17-AAG/carboplatin may effectively overcome this problem.

- **What was the impact on other disciplines?**

Nothing to report.

- **What was the impact on technology transfer?**

Nothing to report.

- **What was the impact on society beyond science and technology?**

Nothing to report.

5. CHANGES/PROBLEMS

- **Changes in approach and reasons for change**

Nothing to report.

- **Actual or anticipated problems or delays and actions or plans to resolve them**

Nothing to report.

- **Changes that had a significant impact on expenditures**

Nothing to report.

- **Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents**

Nothing to report.

- **Significant changes in use or care of human subjects**

Nothing to report.

- **Significant changes in use or care of vertebrate animals.**

Nothing to report.

- **Significant changes in use of biohazards and/or select agents**

Nothing to report.

6. PRODUCTS

- **Publications, conference papers, and presentations**

Journal publications.

1. Young Eun Choi, Chiara Battelli, Jacqueline Watson, Joyce Liu, Jennifer Curtis, Alexander N. Morse, Ursula A. Matulonis, Dipanjan Chowdhury, and Panagiotis A. Konstantinopoulos. Sublethal concentrations of 17-AAG suppress homologous recombination DNA repair and enhance sensitivity to carboplatin and olaparib in HR proficient ovarian cancer cells. Oncotarget 2014 May 15;5(9):2678-87.

2. Liu JF, Konstantinopoulos PA, Matulonis UA. PARP inhibitors in ovarian cancer: current status and future promise. Gynecologic Oncology, 2014 May;133(2):362-9.

3. Konstantinopoulos PA, Wilson AJ, Saskowski J, Wass E, Khabele D. Suberoylanilide Hydroxamic Acid (SAHA) enhances olaparib activity by targeting homologous recombination DNA repair in ovarian cancer. Gynecologic Oncology, 2014 Jun;133(3):599-606.

Books or other non-periodical, one-time publications.

Nothing to report.

Other publications, conference papers, and presentations.

1. Chiara Battelli, Alexander Morse, Hai Hu, Elena Levantini, Gerburg Wulf, Panagiotis A. Konstantinopoulos. HSP90 inhibitor 17-allylamino-geldanamycin enhances sensitivity to double-strand DNA break-inducing agents (platinum and PARP inhibitors) in epithelial ovarian cancer. 2013 AACR Annual Meeting, Washington, DC, 2013.

Website(s) or other Internet site(s)

Nothing to report.

Technologies or techniques

Nothing to report.

Inventions, patent applications, and/or licenses

Nothing to report.

Other Products

Nothing to report

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

- **What individuals have worked on the project?**

Panagiotis Konstantinopoulos: No change

Given that Dr Konstantinopoulos moved to Dana Farber Cancer Institute, this award was transferred to Dana Farber Cancer Institute effective 9/1/2014.

- **Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?**

Nothing to report.

- **What other organizations were involved as partners?**

Nothing to report.

8. SPECIAL REPORTING REQUIREMENTS

Nothing to report.

9. APPENDICES

Supplemental Data

Supplementary Figure 1: Connectivity Map identifies HSP90 inhibitors as candidate compounds that may suppress HR.

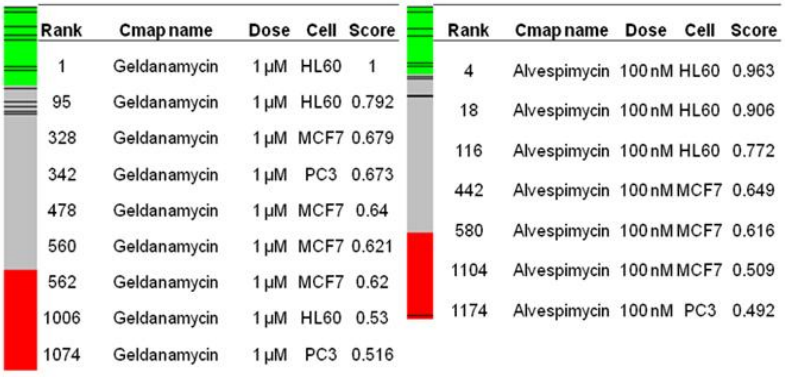
(A) Schematic of the bioinformatics approach used to identify candidate compounds that may suppress of HR. (B) Top ranked compounds, enrichment, permutation p and specificity values as determined by query of the top performing genes of the BRCAness signature. (C) Connectivity mapping of geldanamycin and alvespimycin. The barview is constructed from 6,100 horizontal lines, each representing an individual treatment instance, ordered by their corresponding connectivity scores with geldanamycin (left) and alvespimycin (right). All geldanamycin and alvespimycin instances are colored in black bars. Colors applied to the remaining instances (i.e. gene expression profiles of the cells obtained with other than geldanamycin and alvespimycin) reflect the sign of their scores (green,positive; gray, null; red, negative). The rank, concentration, cell line and connectivity score for geldanamycin and alvespimycin are also shown.

A

B

Cmap Name	Enrichment	P	Specificity
Geldanamycin	0.706	0	0.0163
Alvespimycin	0.63	8E-05	0.0115
Chloropyrazine	0.887	0.0002	0
Meclofenamic acid	0.83	0.0003	0
Fludroxycortide	-0.81	0.0006	0

C



Rank	Cmap name	Dose	Cell	Score
1	Geldanamycin	1 µM	HL60	1
95	Geldanamycin	1 µM	HL60	0.792
328	Geldanamycin	1 µM	MCF7	0.679
342	Geldanamycin	1 µM	PC3	0.673
478	Geldanamycin	1 µM	MCF7	0.64
560	Geldanamycin	1 µM	MCF7	0.621
562	Geldanamycin	1 µM	MCF7	0.62
1006	Geldanamycin	1 µM	HL60	0.53
1074	Geldanamycin	1 µM	PC3	0.516

Rank	Cmap name	Dose	Cell	Score
4	Alvespimycin	100 nM	HL60	0.963
18	Alvespimycin	100 nM	HL60	0.906
116	Alvespimycin	100 nM	HL60	0.772
442	Alvespimycin	100 nM	MCF7	0.649
580	Alvespimycin	100 nM	MCF7	0.616
1104	Alvespimycin	100 nM	MCF7	0.509
1174	Alvespimycin	100 nM	PC3	0.492

Supplementary Figure 2: 17-AAG dose curve in a panel of ovarian cancer cells. (A-C) 17-AAG Dose curves in different ovarian cancer cells. Cells were plated onto a 96-well plate at 1000 cells/well density and treated with indicated concentrations of 17-AAG on the following day. Viability was tested by using CellTiter Glo (Promega) in 5 days.